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Forensic Immunology

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FORENSIC IMMUNOLOGY

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Dr. William C. Boyd of Boston has long been one of the recognized leaders in research on blood groups and the techniques for determining them. His main interest, however, is Immunology, and he now turns to this field and shows how science may contribute mightily to the identification of biological substances and thereby aid the solution of perplexing medicolegal problems. Dr. Boyd is Associate Professor of Biochemistry, Boston University School of Medicine and Special Research Associate, Harvard University, Cambridge, Mass.—EDITOR.

Historical. Tests to identify material as blood, even when dried, have been known for a long time, but they generally depended on properties common to all mammalian blood. Therefore it was extremely difficult, prior to the introduction of immunological methods, to come to a decision in regard to what was often the most vital point of all, namely whether the suspected material was of human or animal origin, or, if of animal origin, which species it was derived from. This decision is now relatively easy, and since this is so, and since immunological methods have been used routinely for a good many years, we tend to forget how great a difference they have made. In the present chapter we shall be concerned precisely with the application of such methods to forensic medicine, and the conclusions which may be drawn.

General. The general principle is easy to state. Animals possess the power of producing protective substances, called antibodies, which may react in various ways with disease producing agents, or products derived from them. In 1897 Rudolf Kraus found that if animals were injected with certain bacteria, one bodily response, particularly after repeated injections, was the production, reaching a peak a week or so after the last injection, of antibodies which appeared in the animal's blood serum. If serum,[1] derived from a sample of these animal's whole blood was mixed with an extract of the bacteria, the originally clear extract became cloudy and often a precipitate was produced. This was the discovery of the precipitin reaction.

It is not only agents of disease which can stimulate the formation of antibodies, however, for many other things, most proteins from a species foreign to the animal injected, particularly, may do so. Thus the injection of albumin from hen's egg (a substance in itself perfectly harmless) into rabbits causes them to produre anti-egg albumin antibodies.

[1] When blood clots, a clear yellowish fluid separates. This is the serum. It contains the inorganic and organic non-protein constituents of blood, and many of the protein constituents, including the substances (defined below) called antibodies.

Antibodies are of various kinds; all of them, including the precipitins which will be of particular interest to us here, possess one distinguishing characteristic which gives them their forensic usefulness, namely, their specificity. Antibodies produced in an animal are directed particularly against the foreign protein injected, thus antibodies produced to antigens[2] derived from species A do not generally react[3] positively with antigens of species B, and vice versa, unless A and B are more or less related. This provides us with a way of identifying the species origin of various materials which may come up for medicolegal examination, and is the basis of forensic immunology.

Antibodies have received various names depending on their mechanism of action. (1) If they dissolve the antigen in test suspensions, they are called lysins; (2) If they precipitate the antigen, they are called precipitins; (3) If they cause the particles of antigen to agglutinate (clump or adhere), they are called agglutins, etc. Some of these may be different from the others but it is very likely that in some cases one and the same antibody is capable of acting in various ways, depending on circumstances.

In passing, we may mention that the compound formed when antibody and antigen react has an affinity for certain other components of the blood which are called collectively complement. Unless an excess of complement is present, the occurrence of an antibody-antigen reaction uses up all the complement. This is called complete fixation. By suitable test, it can be demonstrated that complement has been "fixed" from a mixture, and that an otherwise perhaps undetectable antibody-antigen reaction has taken place. If a certain antigen was known to be present in the mixture, the fact of its reacting and thus fixing complement indicates that the corresponding antibody was present. This principle is made use of in the Wassermann test for syphilis. Similarly, if a known antibody were present, the fact of complement fixation may indicate indirectly the presence in suspected material of specific antigen.

Applications. One of the earliest applications of the precipitin reaction was the differentiation of horse meat from the flesh of other species (Uhlenhuth and Jesz), and later the test was applied to differentiate the bloods of various species. The most complete treatise on precipitin reactions of blood is the classic book by Nuttall.[4] The precipitin test may also be used to detect the presence of bacterial antigens and thus in some

[2] This is the name given to substances stimulating the animal to the production of antibodies.

[3] By react we mean combine or affect in a visible way, or a way which can be detected indirectly.

[4] Nuttall, G. H. F.: *Blood Immunity and Blood Relationship*, Cambridge, The University Press, 1904.

cases the presence of a certain disease. A good illustration of the forensic application of such tests is the use of the "thermo-precipitin test" of Ascoli[5] to demonstrate the presence of a heat-stable[6] antigen of the anthrax bacillus.[7] Because of the public health aspect of anthrax (infections resulting from skins, hair, etc. of diseased animals), this procedure is of considerable importance, especially abroad.

The possible applications of immunology to forensic medicine are numerous. We list a few in Table I.

TABLE I

APPLICATIONS OF IMMUNOLOGY

Antigen	*Demonstrated by reaction of*	*Possibly significant in cases of*
Human blood protein	Precipitation or complement fixation	Murder
Human blood (from bits of tissue)	Precipitation or complement fixation	"Hit-and-run" automobile cases, etc.
Human blood (from bones)	Precipitation or complement fixation	Murder
Human seminal fluid	Precipitation or complement fixation	Attempted rape
Anthrax antigen	"Thermo-precipitin test"	Disease from raw materials (skins, etc.)
Human blood group antigen A or B	Absorption of agglutinins	Murder (Identification of blood group of dried stain)
Various bacteria	Agglutination	Certain diseases (typhoid, etc.)
Muscle proteins of certain animals	Precipitation	Violation of food or game laws

We may divide the material to be taken up in the present chapter into two parts. In the first part we shall consider the technic of the precipitin reaction, using this as a typical example of an immunological reaction which has forensic application. It will be wise to include also a study of the difficulties and pitfalls which the inexperienced may encounter. In the second part we shall take up the question of how the court may recognize the proficiency and competence of an expert who presents immunological data as evidence or who claims to be qualified to carry out such tests.

PART I: METHODS

The basic principle of good forensic immunological technic may be summarized by stating that the expert must in all cases assure himself of the specificity of all antisera[8] he employs in a medicolegal case. The blood serum from a rabbit which has been injected several times with human blood will generally

[5] Ascoli, A.: *Die Thermopräzipitin reaktion*, Wien und Leipzig, Josef Safar, 1922.

[6] In immunology, heat stable means resistant to heating to 55-60°C. for 30-60 minutes.

[7] An acute infectious disease. It is essentially a disease of the lower animals, especially cattle and sheep, but may be transmitted to man by contact with the bodies of infected animals.

[8] The serum of an animal containing antibodies.

give a precipitate when mixed with human blood or an extract of material which has been stained with human blood. However, any student of evolution will realize at once that such a serum might be expected to give a precipitate (less in amount, of course) also with blood from the anthropoid apes, and indeed, this is generally the case. To a still smaller extent, certain sera may cross-react also with blood of other mammals. Table 2, taken from Nuttall's book, will serve as an illustration. Unless these facts are taken into account, an erroneous

TABLE II

Relative amounts of precipitate with 3 different anti-human blood rabbit sera and equal amounts of blood serum of various species.[4] Amount of precipitate obtained with human blood taken arbitrarily as 100.

Species	Immune Serum		
	1	2	3
Man	100	100	100
Chimpanzee	—	—	130*
Gorilla	—	—	64
Orang-utan	47	80	42
Cynocephalus moron	30	50	42
Cercopithecus petaurista	30	50	—
Ateles vellerosus	22	25	—
Cat (Felis domesticus)	11	—	3
Dog (Canis familiaris)	11	—	3

* Loose precipitate.

diagnosis of human blood could be made when in fact the unknown blood sample came from an animal. The expert, however, will avoid error through his knowledge of several facts:

(1) A good antiserum will practically always react with the homologous[9] antigen, even when the latter is in a highly dilute solution. Cross reactions, that is, reactions with material from other species, in general will occur only with more concentrated solutions of the unknown antigen being tested. By including suitable controls[10] in the tests, these differences may be made apparent.

(2) It is generally possible to remove from an antiserum the antibodies which are responsible for the cross reactions, leaving a reagent which is completely specific or nearly so. The technic of this is discussed below.

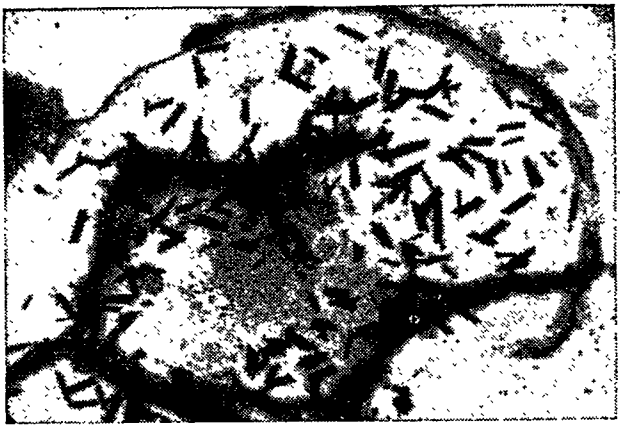
Preliminary. Naturally we shall not attempt to determine the species origin of blood in suspected material unless previous tests have established the presumption that some sort of blood is really there. The benzidene test is one of the most sensitive, but is not specific for blood; a positive benzidine re-

[9] The antigen injected.

[10] By controls we mean tests set up with known materials, so that we may anticipate the outcome, positive or negative, as the case may be. They serve to establish that reagents have that activity, and only that, which is desired.

action[11] can only be considered presumptive evidence. A positive hemochromogen[12] or Teichmann[13] test will show definitely

Fig. 1. *Hemin crystals (Teichmann) above. Hemochromogen crystals, below.*



that blood is present. See Figure 1. Once this fact is confirmed, the qualified investigator is ready to perform precipitin tests

[11] The benzidine test is performed as follows: To 3 cc. of a saturated solution of benzidine in glacial acetic acid add 2 cc. of the solution to be tested and 1 cc. of 3 per cent hydrogen peroxide. A positive reaction is indicated by the development of a blue color.

[12] Hemochromogen test. Probably the best results in obtaining these crystals are given by the Takayama method. Takayama's solution is as follows:

Sodium hydroxide (10 per cent)	3 cc.
Pyridine	3 cc.
Glucose (saturated solution)	3 cc.
Distilled water	7 cc.

This solution when kept in a yellow glass-stoppered bottle, will re-

to determine whether the blood be human or from an animal source, and if the question is material, from what animal.

Technic of the Precipitin Test. A precipitating serum, if mixed with the homologous antigen in the proper proportions, will produce a clouding of the originally clear fluids, usually followed by the formation of a flocculent precipitate. The reaction is more easily seen, and takes place over a wider range of concentrations, if it is carried out by placing one fluid (the antigen) over the other (the antiserum) in a suitable test tube. By this interfacial technic the reaction is visible as the formation of a white zone or plane at the junction of the two clear fluids. This is often called the "ring" test. See footnotes [14, 15, 16, 17, 18, 19, 20, 21].

Reagents. The reagents involved are two: antiserum and antigen. Both should be crystal clear (though not necessarily colorless). If not clear, they should be filtered through a retentive filter paper, and if necessary centrifuged[42] a long time at high speed.

Preparation of Antiserum Material for Injection. Precipitins suitable for differentiating human from other blood may be produced by injecting rabbits with isotonic[22] laked whole[23] human blood, or by injecting defibrinated whole blood,[24]

main effective for a month or two. Two or three drops of the above solution are added to some of the powdered material, from the stain, on a microscope slide which is later examined under low power. Gentle warming of the slide may hasten the reaction. If blood is present pink feathery crystals of hemochromogen or reduced alkaline haematin, usually in sheaves, clusters, or in slightly varying formation, will be seen.

[13] Teichmann's test. Place a very small drop of blood on a microscopic slide, add a small drop of water and stir to lake the blood. Add a fraction of a drop of dilute (0.9 per cent) sodium chloride solution and carefully evaporate to dryness over a low flame. Put a cover glass in place, run underneath it a drop of glacial acetic acid and warm gently until the formation of gas bubbles is noted. Add another drop of glacial acetic acid, cool the preparation, examine under the microscope.

[14] Boyd, W. C.: *Fundamentals of Immunology,* New York, Interscience Publishers, Inc., 1943.

[15] Glaister, J.: *Medical Jurisprudence and Toxicology,* Baltimore, Williams and Wilkins, 1942.

[16] Hektoen, L.: The Precipitin Test for Blood, *J.A.M.A.*, 70, 1273, 1918.

[17] See footnote 5.

[18] Schiff, F. and Boyd, W. C.: *Blood Grouping Technic,* New York, Interscience Publishers, Inc., 1942.

[19] Smith, S. and Glaister, J.: *Recent Advances in Forensic Medicine,* Philadelphia, Blakiston's Son and Co., 1931.

[20] Uhlenhuth, P. and Steffenhagen, K.: Die biologische Eiweiss-Differenzierung mittels der Präzipitation unter besonderer Berücksichtigung der Technik, Handbuch der Pathogenie Mikrooganismen, 3, 257, 1913.

[21] Uhlenhuth, P. and Seiffert, W.: Die biologische Eiweisz-differenzierung mittels der Präcipitation mit besonderer Berücksichtigung der Technik, *Handbuch der Pathogenic Mikrooganismen,* 3, 365, 1913.

[22] Brought to the same osmotic pressure. If soduim chloride (common salt) is used, this means bringing the concentration to 0.9 per cent.

[23] Treated so as to dissolve the red cells and release the hemoglobin from them.

[24] Blood prevented from clotting by removing the clot as it forms, as by whipping with an instrument such as an egg-beater or shaking with glass beads.

plasma,[25] or serum.[26] Serum is easiest to procure, and seems on the whole as satisfactory as anything else. Before injecting the serum it is sometimes diluted (with 0.9 per cent soduim chloride solution) to an approximately 1 per cent protein content (i.e., dilute 1:6 or 1:7). Several injections are necessary; the present writer injects each animal 3 times a week for 3 or 4 weeks before taking a bleeding.

Apparatus. It is convenient to make the antigen dilutions (0.9 per cent sodium chloride solution is used for diluting fluid) in test tubes such as the 13 x 100 mm., "Wassermann tubes," using ordinary serological pipettes.[27] The reaction may be carried out in test tubes of about 9 x 110 mm., putting in 0.9 cc. of antigen dilution, and 0.1 cc. of antiserum (Uhlenhuth[28, 29]). Much material may be saved and the reaction ren-

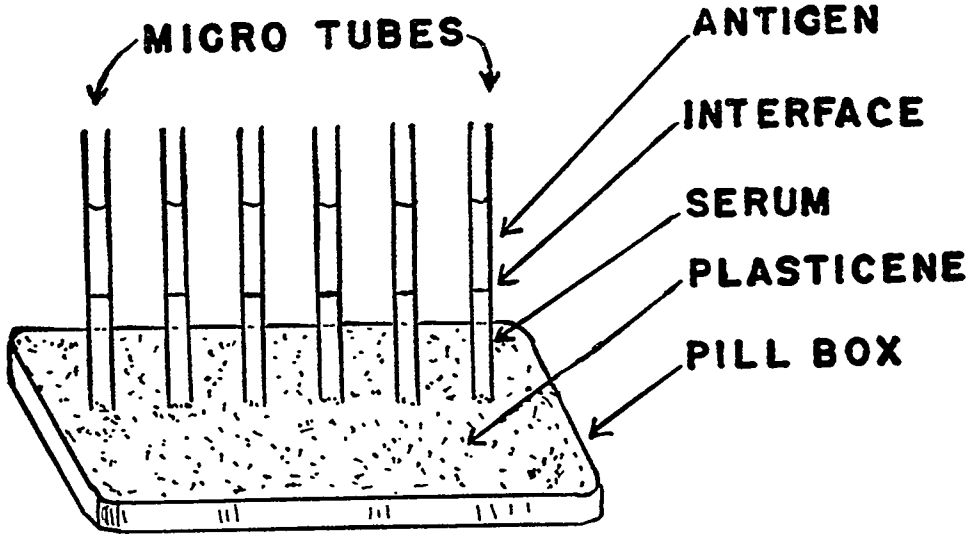


Fig. 2. Diagrammatic representation of set-up for the Precipitin Test.

dered easier to read, by using "micro" tubes made from semi-capillary tubing, about 35 mm. long and 2.5 mm. internal diameter. In these there is placed about 0.4 mm. of antiserum and on top of this about 0.04 cc. of antigen dilution.[30]

For the Uhlenhuth tubes, special racks may be used in which the tubes hang from their lip, and the bottom, in which the reaction takes place, is free. The "micro" tubes may be supported by sticking their lower ends into plasticene in a small shallow container, such as the metal cover of a pill box. Or

[25] The fluid part of blood, prevented from clotting by the addition of an anticoagulant, with the cells removed.
[26] See footnote 1.
[27] Graduated tubes designed for delivering accurately measured small volumes. Serological pipettes are graduated all the way to the tip.
[28] See footnote 20.
[29] See footnote 21.
[30] See footnote 14.

special small racks of wood or wire may be constructed, which have the advantage of leaving the bottom of the tube open to inspection, and of not soiling the outside of the tube.

For use with the small tubes, fine capillary pipettes, made by drawing ordinary laboratory glass tubing to a point in a flame, and small rubber bulbs (2 cc. capacity) will be needed. With a little practice, it is easy to transfer with these the desired amount of fluid into the little precipitin tubes and obtain the desired sharp line of junction.

Determination of Titer of Antiserum. In order to be able to gauge the significance of a positive reaction, it is of course necessary to have a fair idea of the potency of the antiserum or antisera being used. It seems sufficient to estimate the strength of the antiserum by determining the highest dilution of antigen which is precipitated by the antiserum.

The antiserum is tested with successive dilutions, for example 1:10, 1:100, 1:1000, etc., dilutions of the homologous antigen (i.e. human serum), and the last dilution noted which gives a visible reaction in 20 minutes or less. This is the titer of the antiserum. If desired, dilutions in smaller steps, such as 1:5, 1:25,... 1:3, 1:19..., may be tested.

A serum with a titer of 1:1000 or over is generally considered satisfactory. However, the amount and density of precipitate, and the rapidity of the reaction, are also of significance. An antiserum with a titer of at least 1:20,000 is superior, and to be preferred because this means it is more sensitive and will detect smaller quantities of antigen and with a more striking positive reaction.

The tests are set up by transferring about 0.04 cc. of antiserum to each of 6 or 7 of the small tubes described above, using the capillary pipette and rubber bulb. Serum in one of the tubes is overlayed with saline, for the control, and no visible change should occur in this tube. Then with another capillary pipette, or with the same pipette, thoroughly rinsed, there is placed over the serum in another tube and in contact with it so as to form a sharp junction without bubbles, about 0.04 cc. of another of the antigen dilutions in the other tubes, and so on, keeping the tubes in order in the support, or marking them to prevent confusion. One may begin with the most dilute solution and progress to the most concentrated, without rinsing. A control consisting of the strongest concentration of antigen overlayed with saline is also desirable, and no visible change should occur in this tube.

Characteristics of a Positive Reaction. If the above technic is used, and the antigen being tested is human blood, a positive reaction will result, consisting of a thin white disk or plane at or very near the junction of the antiserum and antigen

which appears within a few minutes after the antigen has been added, and grows heavier. In a very strong reaction, particles of precipitate may begin to fall down through the antiserum. The disk of precipitate should remain horizontal if the tube is tipped, showing that it is not dirt or other contamination attached to the walls of the tube. Every precaution, naturally, is taken beforehand to ensure the cleanliness of the tubes.

The reactions should be read in a good light, with a dark background behind the tubes. A well lighted window which has a cross bar which can be used for the background is very satisfactory. Fluorescent illumination with a dead black background is good.

The tests are carried out as described immediately above. Reactions are to be judged positive when they fulfill the conditions laid down there. If no visible precipitate, of the character described, materializes the test is negative and the suspected blood being tested, did not come from a human being.

Test of Specificity of Antiserum. Before use in practice, each antiserum must be tested for specificity.[31] If it is to be used in America or Europe in the usual type of medicolegal case, it will probably be sufficient to ascertain that it does not react with any of a series of dilutions of the blood of common domestic animals: dog, sheep, pig, cat, chicken, goose, etc. If it is desired to differentiate between human blood and blood of anthropoid apes and monkeys, which is seldom, it will be necessary to test the antiserum with these bloods also. It is probable that a reaction will be found with the blood of the higher apes. In some cases also a reaction will be obtained with (low dilutions only) various mammalian bloods (mammalian reaction of Nuttall.[32]) The cross reactions to be looked out for naturally will vary with the locality and the circumstances.

Production of a Completely Specific Antiserum. In case the antiserum is found to precipitate with any of the non-human samples tested, it may be rendered specific by absorption. The principle of this is as follows: Let us call any antigen, differing in species origin from the antigen injected, heterologous. Some of the reactive heterologous antigen is added to the antiserum, the precipitate is removed and the absorbed antiserum is thus deprived of its power to react with this heterologous material.

The procedure of specific absorption is described in various books and papers[33, 34, 35, 36].

[31] Unless the reagent is specific for the substance it is designed to detect, a positive reaction will not necessarily denote the presence of that substance.

[32] See footnote 24.

[33] See footnote 14.

[34] See footnote 18.

[35] See footnote 20.

[36] See footnote 21.

Preparation of the Unknown Specimen for Test. Before the reaction can be carried out, the unknown material must be brought into solution. If the blood stain is on hard material such as glass, smooth wood, metal, etc., it should be scraped off, powdered, and the powder extracted.[37] A roughly equal amount of scrapings (if any can be obtained) from the unstained substrate[38] should be extracted in the same way for use as a control. If the stain is on cloth, paper, etc., the stain, or a portion of it if it is large, should be cut out and extracted. An equal portion of unstained material should be extracted at the same time. In some cases, as in checking stains from a possibly innocent man's clothes, one may have to buy a new suit (always an expensive one!) if a piece is cut out of the material. If a few threads are pulled out here and there from the stain, and a few for controls from unstained areas, a test, at least of preliminary nature, can be made without such removal of test material being noticed.

The material is extracted with physiological saline (0.9 NaCl solution), overnight in the icebox. When plenty of material is available, it is suitable to use about 1 cc. of saline (more if the material is very absorptive) for each square centimeter of stained material, in the case of cloth, etc. or for each 4 to 5 mg. of dried blood powder. This should give a solution, if all the dried serum proteins dissolve, roughly equivalent to a 1:10 or 1:20 dilution of fresh serum. The actual content of the dissolved protein should be estimated, by shaking portions of the various dilutions to produce foam, and by placing small amounts of them over concentrated nitric acid in little tubes, and noting the greatest dilution of the extract which gives a reaction;[39] this will correspond to a dilution of about 1:1000 of fresh serum. The dilution which just forms stable foam is similar, also corresponds to about 1:1000 (Uhlenhuth[40,41]).

In some of the most important cases the amount of blood stain obtainable is not as great as just recommended, as when a suspected knife has been cleaned, and only a trace of material suspected of being blood remains in the nail groove. In such cases the extraction may have to be made with 0.1 to 0.2 cc. of saline solution.

The extract should be filtered, if possible, and if necessary centrifuged at high speed to clarify it.[42]

[37] Mixed with solvent (usually 0.9 per cent salt solution) and allowed to stand, preferably in the cold.

[38] The material bearing the stain.

[39] A positive reaction is the formation of a white or yellow disk ("ring") of precipitate at or near the junction between the solution and the acid.

[40] See footnote 20.

[41] See footnote 21.

[42] Spun rapidly around an axis in one of the devices called centrifuges. By thus subjecting the specimen to the action of an "artificial gravity," sedimentation of particulate matter present is hastened.

Over the serum will be placed, in five of the antiserum-containing tubes, a 1:100,000, a 1:10,000, a 1:1000, a 1:100, and a 1:10, respectively, dilution of the extract of the unknown stain. (When material is very scanty, or the extract very diluted, the highest dilutions may be omitted.) For this the same pipette may be used, beginning with the most dilute solution and progressing to the most concentrated, without rinsing. When the 1:100 dilution is reached, some should also be placed over normal serum. Then with separate pipettes, saline is placed in another antiserum-containing tube, and an extract of the unstained substrate[43] in another, in that order. With another pipette a 1:1000 dilution of some heterologous blood serum[44] is added to an antiserum-containing tube, and a 1:1000 dilution of known human serum is placed in another tube also containing antiserum. The whole set-up is shown in figure 3 and Table III on the following page.

Purpose of the Controls. In order to be able to make positively the diagnosis of human origin of unknown material, the controls as outlined above are needed, *viz*: *Normal rabbit serum*: this is used to test if the extract of the unknown material will give a (non-specific) precipitate with sera not containing anti-human precipitins. Extracts of some material have a non-specific precipitating power when mixed with sera. The reaction in this tube must be negative. *Saline*: This tests if the antiserum tends in general to cloud when in contact with other, non-specific fluids. This tube also serves as a standard with which to compare the appearance of the other reactions. The reaction here must be negative. *Substrate*: (tube 8) this tests if the substrate has, independently of the stain, any specific or non-specific precipitating power. The result here must be negative. *Heterologous blood,* (tube 9): this controls the specificity of the antiserum, and shows that it does not react with mammalian bloods in general,[45] at least not in high dilution. The reaction here must be negative. *Known human blood,* (tube 10): this controls the activity of the antiserum. It is necessary to show that the serum still reacts well. This tube also serves as a standard reaction with which to compare any positive reaction found with the unknown.

These controls are of great importance; no one of them should be omitted.

Evaluation of the Results. If the controls are satisfactory (tubes 6 to 9 negative, tube 10 positive), positive reactions appearing in tubes 1, (1:100,000), 2, (1:10,000), 3, (1:000),

[43] See footnote 38.
[44] See footnote 1.
[45] In case the results are to be presented in court, the records may be more impressive if several heterologous bloods are tested.

TABLE III

SCHEME FOR THE COMPLETE PRECIPITIN TEST, WITH CONTROLS

	Tube Number									
	Tests					Controls				
	1	2	3	4	5	6	7	8	9	10
Upper Layer	extract, 1:100,000	extract, 1:10,000	extract, 1:1000	extract, 1:100	extract, 1:10	extract, 1:100	0.9% NaCl	substrate extract	heterologous blood, 1:1000	known human blood 1:1000
Lower Layer	anti-serum	anti-serum	anti-serum	anti-serum	anti-serum	normal rabbit serum	anti-serum	anti-serum	anti-serum	anti-serum
Expected Result						—	—	—	—	+

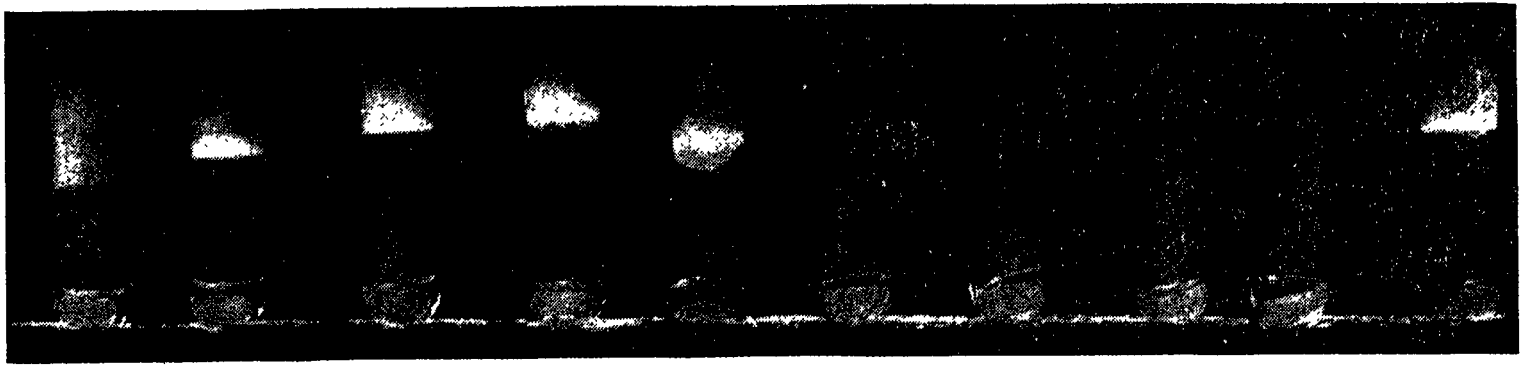


Fig. 3. *Photograph of the Precipitin Test.*

or 4, (1:100), indicate that the blood stain was of human (or anthropoid) origin. A reaction in tube 5, (1:10), alone is of less significance,[46] unless the nitric acid test shows the original extract to have been unusually weak. A reaction in tube 4, (1:100), alone is probably significant, but shows the concentration of serum proteins (antigens) to be very low in the extract. In a clear cut case, positive reactions will be obtained in tubes 5,4,3, and possibly 2 and 1.

Limits of Applicability of Test. A positive reaction, as described, with satisfactory controls, is diagnostic of human (or ape) blood. Human blood can be differentiated from anthropoid blood by using a good antiserum, specifically absorbed. The likelihood of finding ape blood in an American murder case is of course pretty remote. Human blood stains 15 to 60 years old have been identified by the precipitin test.

A verdict in the opposite sense, i.e., that a stain is not human blood, should be ventured only if the stain is in a good state of preservation, not too old, and preferably only if a good reaction can be obtained on testing it with an antiserum to blood of some animal species, thus positively identifying it.

In case the extract of the unstained substrate causes a clouding, a confident diagnosis will be impossible. Sometimes neutralizing the extract, (i.e., bringing it to Ph7[47]) may remedy this. If the extract is highly dilute, however, neutralization seldom helps.

Other Applications to Legal Medicine. There is not enough space here to discuss the technic of other serological reactions which have medicolegal applications. Some have been mentioned above. Descriptions may be found in some of the following references [48, 49, 50, 51, 52, 53, 54, 55, 56]. Strictly, blood grouping also falls in the category of serological technic[57] and the legal aspects of these tests will be found described elsewhere.[58]

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[46] Particularly if the antiserum used is known to precipitate low dilutions of certain heterologous bloods.

[47] pH represents a measure of acidity, and a value of 7 indicates the acidity of pure water, i.e., neutrality.

[48] Ascoli, A.: *Die Thermopräzipitinreaktion,* Wien und Leipzig, Josef Safar, 1922.

[49] See footnote 14.

[50] See footnote 15.

[51] Gonzales, T. A., Vance, M. and Helphern, M.: *Legal Medicine and Toxicology,* New York, D. Appleton-Century Co., 1937.

[52] Lucas, A.: *Forensic Chemistry and Scientific Criminal Investigation,* London, Edward Arnold and Co., 1935.

[53] See footnote 18.

[54] See footnote 19.

[55] See footnote 20.

[56] See footnote 21.

[57] Serology may be defined as the science of reactions involving the use of serum.

[58] Chapter ..., Boyd, W. C.: *Protecting the Evidentiary Value of Blood Group Determinations,* p....

PART II: EXPERT QUALIFICATIONS

Criteria of Expertness in Forensic Immunology. The exact place of the immunological expert, or indeed of any expert, is not always too clear in legal procedure in this country[59, 60]. In most jurisdictions, the question whether a witness qualifies as an expert is decided by the presiding justice. The relative weight to be given to his evidence is left to the decision of the jury. In theory, the expert merely presents evidence which could not have been found out by laymen, but laymen, (the jury) are left to draw the vital conclusions from this evidence. In practice, it not infrequently happens that the expert (or one of his colleagues) has to indicate by his opinion what sort of conclusions are allowable from certain scientific data, for he and other expert witnesses in the case constitute the chief source of erudition for court and jury in respect to criteria of Scientific Proof.

It is important that the judiciary and the members of the legal profession should be able to form an opinion as to the qualifications of any one claiming to be an expert, or suggested as being capable of performing tests in forensic immunology. Unless in the future some scientific organization will undertake to certify individuals as being qualified in this capacity, the decision will always remain a matter of some difficulty, since members of the legal profession cannot be expected to know all the technical details involved. It is quite possible for a pseudo expert, possessing a facile mind and superficial knowledge of the literature on the subject, to present testimony even more apparently convincing than a genuine expert. However, certain considerations will aid in forming an opinion and we may discuss some of them here.

(1) The first and most important qualification, probably, is experience. This means that the individual should have been engaged in making forensic immunological tests or carrying out research in immunology for some time, certainly at least for a year. By proper questioning as to the number and types of tests the individual has carried out, it should be possible to form an estimate of his competence.

(2) An expert in forensic immunology should preferably hold some advanced scientific degree such as M.D., Ph.D., etc. Some individuals, of course, without such degrees, are entirely qualified in this field and the absence of such scholastic attainment should not automatically disqualify a person. Conversely, the possession of such a degree is by no means proof of the competence of an individual. The degree of Ph.D. may be

[59] Smith, H. W.: Scientific Proof and Relations of Law and Medicine, *Univ. of Chi. L. Rev.* 10:243, April 1943.

[60] Williams, E. H.: *The Doctor in Court*, Baltimore, Williams and Wilkins Co., 1929.

obtained in a wide variety of subjects, some of them entirely unrelated to immunology. Most medical schools offer some training in immunology to their students, but it is probably not realized by the layman how brief and inadequate is the acquaintance of the average doctor with this subject. Even if he remembered everything he had been taught, the doctor fresh from medical school would not be qualified, without further experience, as an expert in immunology.

(3) It is preferable that the individual should do the tests himself with his own hands. However, tests carried out by the properly trained assistants, provided they are supervised and checked by the supervisor of the laboratory, are sometimes accepted. I do not mean to go into the legal side of this. In any case, the person offering the tests as evidence should have carried out such tests himself at *some time,* that is, his knowledge must not be purely book knowledge, and he must be able and willing personally to vouch for the accuracy of test results he describes on the witness stand.

(4) The expert should show an adequate acquaintance with the scientific literature of the subject. The number of books on immunology is very great and the witness cannot be expected, of course, to remember or know, all of them or even any one of them in complete detail. He should, however, know the names of some of the books, be able to give a rough idea of their contents, and should be able to describe the technic he used in his own tests in a detailed way, pointing out any significant departures from customary procedures as described in the standard texts on the subject.

(5) While it is not absolutely essential, it is highly desirable, that an individual claiming to be an expert should have published results or original individual research of his own dealing with some phase of immunology. In the present state of relations between law and medicine, the publication of original research must be regarded as important proof of outstanding mastery of a field. Whether this indicates competence in the particular field in which evidence is being offered, is sometimes another question and not always easy to decide.

(6) Finally, perhaps one of the best criteria is whether or not the individual is known and recognized as qualified by others in the same general locality who also work in the field. Essentially the field is a small one, and news of mistakes tends to travel.

Signs of the Pseudoexpert. In the absence of any authoritative group authorized by the several states to undertake the routine certification of experts in immunology, there always remains the possibility that grossly incompetent persons may attempt to present to the court evidence based, or supposedly

based, on immunological tests. Indeed, certain facts suggest that this not infrequently occurs. There are considerations which aid the court in detecting such pseudoexperts. (1) Absence of proper experience in the field, or at least in some branch of immunology. (2) Abscence of any background of proper scientific training is always suggestive but not conclusive. (3) Lack of knowledge of books dealing with the subject and of living research workers who have recently published on the subject. Of course a pseudoexpert may claim to know the books in the field. It is important that he be able to suggest by his replies some degree of acquaintance with their contents and the scientific position of the men who write them. (4) Failure to have published any original research (as indicated above, this is not conclusive by any means). (5) Evidence that the individual in question is unknown to other persons who seem on reliable evidence to be real experts in the field. In practice this is probably the most suspicious circumstance.

Things an Expert Should Do to Insure His Tests the Utmost Probative Value

(1) He should identify and store samples entrusted to him in such a way that no question of mistakes in identity can later arise. The place where they are stored should be accessible only to trusted members of his laboratory, or else the samples should be in a locked box.

(2) He should keep the samples (the perishable ones in the ice box) long enough after the tests have been completed to make sure that no retesting will be required on following days.

(3) Samples of the sera used in making the tests should be preserved for possible checks as to their strength and specificity in case of any doubt subsequently arising.

(4) If the tests seem to give doubtful results, or if any special difficulties present themselves in making the tests or in interpreting the results, the expert will repeat the tests until he is satisfied of the entire accuracy of the results; if necessary, will even ask a second accredited expert to test the samples independently.

(5) In some cases it may be necessary for an expert to outline to his lawyer the proper questions to ask him in order that he may be qualified as a competent expert in the eyes of the court. Attorneys who have not previously dealt with such cases are not always aware of certain pitfalls. If the expert is not a physician he must prepare his own attorney for the attempt which may be made by the opposing counsel to discredit him because of this fact.

(6) The expert should guard himself, while presenting his evidence against a possible attempt of the opposing attornev to

make the whole question seem so complicated and involved that the court loses all confidence in it. The principles involved are essentially simple, and can be so presented. The way in which perfectly valid scientific evidence can be made to seem doubtful or even downright wrong is well illustrated by the passage quoted from Zinsser in reference 58.

A legal colleague of the writer has said, apropos of the foregoing:

"If I were cross-examining the expert in a serious case, I would most assuredly find many loopholes by which I could more or less successfully trip him into either contradicting himself, or impliedly or expressly admitting that his conclusion is by no means sufficiently certain to sway the verdict in favor of the side he is supporting. The laboratory tests, as shown in the treatise, are complex and admit of more than one interpretation, [with this statement I emphatically do not agree. W.C.B.] even if followed through precisely as suggested. The several interpretations that may result from the tests applied are in themselves a sufficient argument against the adoption of the expert's opinion.

".... expert testimony in any other field, such as handwriting and especially in psychiatry, is always conjectural to a large extent. If this element of conjecture, and such an element is definitely present in the tests enumerated in the treatise, can be shown to be present in medical testimony based upon scientific experiment, then it is fatal to the issue. This is especially true in scientific opinion, which by definition, must be most exact in its conclusion.

"In this opinion I am especially mindful of the murder case which was tried in .... County several years ago, in which a young assistant district attorney, with only an elementary knowledge of chemistry, so confused a nationally famous expert that he retired after several days of testimony in utter confusion."

If the expert is really properly qualified he should never allow this sort of thing to happen. His knowledge of his subject is, in all probability, enormously greater than that of any other person in the courtroom; his confidence in replying to questions should correspond. With all deference to my legal friend, I submit that in conclusions from tests such as outlined above there is nothing that can properly be called conjectural, unless he wishes to say that my statement that 10 pounds of dry TNT, activated by the detonation of a good charge of mercury fulminate placed in its center, will produce quite an explosion, is "conjectural." If he has any doubt, let him prove it; either by sitting on top of the TNT, or by sending me two samples of cloth, one stained (in the presence of reliable wit-

nesses) with human blood, and another similarly stained with pig blood!

As to the danger of contradicting oneself; with a really competent expert it should never happen, if he remembers one simple rule: always answer each question correctly. The story of the tests themselves, being consistent in itself, cannot be made contradictory. Of course the expert must be careful that he answers in such language that his meaning is understood; otherwise apparent contradictions may arise.